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(71) Applicant *(for all designated States except US)*: MERCK & CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: HOLLIS, Gregory, F. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). PATEL, Myur, D. [GB/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).

(74) Common Representative: MERCK & CO., INC.; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).

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(57) Abstract

The present invention relates to DNA molecules endocing a canine IgE and species-specific regions of the canine IgE constant region. The invention comprises the DNA molecules, proteins encoded by the DNA molecules, antibodies to the proteins, cells transformed by the DNA molecules, assays employing the transformed cells, compounds identified by the assays and kits containing the DNA molecules or derivatives thereof.

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TITLE OF THE INVENTION
DNA ENCODING CANINE IMMUNOGLOBULINS

CROSS-REFERENCE TO RELATED APPLICATIONS

This is a continuation of U.S. Serial No. 08/336,583, filed on November 9, 1994,now pending, and a continuation of U.S. Serial No. 08/336,891, filed on November 9, 1994, now pending.

BACKGROUND OF THE INVENTION

This invention describes cloning and characterization of the canine IgE gene. The canine IgE gene was isolated using a human IgA constant region probe to clone a piece of the dog genome. IgA-containing cloned fragments of the dog genome were searched for IgE-related sequences. The identified areas were characterized in detail by nucleotide sequence analysis. This invention provides specific sequence information which permits targeted modulation of IgE-mediated immune responses.

The invention relates to DNA molecules encoding a canine IgE and species specific regions of canine IgE constant region. The invention comprises the DNA molecules, proteins encoded by the DNA molecules, antibodies to the proteins, cells transformed by the DNA molecules, assays employing the transformed cells, compounds identified by the assays and kits containing the DNA molecules or derivatives thereof.

Traditionally, hypersensitivity responses in the dog have been controlled by corticosteroid therapy which has adverse metabolic effects and produces generalized immunosuppression. The cloning and sequence determination of the canine IgE gene permits novel approaches to the control of IgE-mediated hypersensitivity reactions by facilitating targeting of the IgE molecule and its interaction with the IgE receptor. These approaches include, but are not limited to eliciting an immune response directed at specific peptide epitopes present in canine IgE to control allergic reactions and using the canine IgE sequence as part of a

screen to identify small molecules that alter IgE mediated responses to allergens.

Immunoglobulin (Ig) proteins consist of two identical light (L) chains and two identical heavy (H) chains. Both Ig L and H chains contain an amino-terminal variable region of approximately 110 amino acids that forms the antigen binding domain. The carboxy terminal constant (C) region domains of each chain is defined by two isotypes of IgL chain (kappa and lambda) and multiple isotypes of IgH chains (mu, delta, gamma, epsilon and alpha which define IgM, IgD, IgG, IgE, and IgA, respectively). The IgH chain C regions contain the effector functions common to antibodies of a given isotype.

IgE antibodies are responsible for mediating allergic responses. IgE binds to mast cells through an Fce receptor and, when cross-linked by binding antigen, triggers a cascade of events that leads to the release of allergic mediators. Because of the central role that IgE plays in mediating allergic reactions, the region of the Igε constant region involved in Fcε receptor binding is of great interest. Inhibition of binding of IgE to its receptor on mast cells may be a way to control allergic responses.

Interestingly, of all five isotypes of immunoglobulin, the sequence of the Igε C region is the least well conserved across species. Consequently, studies of allergic reactions in a specific species are aided by having the primary amino acid sequence available for the Igε C region gene of that species.

The IgE antibody class plays a central role in type I immediate hypersensitivity. IgE binds to specific high-affinity receptors on mast cells and basophils and, when associated with antigen, triggers degranulation of vasoactive substances to produce allergic reactions. Because of its role in allergy, substantial effort has been made to understand how the Igε C region (which defines IgE) interacts with the Fcε receptor on mast cells and basophils to trigger degranulation upon binding antigen. These studies indicate that binding to the Fcε receptor reside in the Igε CH3 and CH4 domains. Additional studies have used linear peptides to map the Igε binding site. In one of

these studies, an octapeptide from the human Igε gene (Pro345-Phe-Asp-Leu-Phe-Ile-Arg-Lys352) inhibited passive sensitization, presumably by occupying the Fcε recetor sites on cells (Nio et. al. 1993). The equivalent region of the canine Igε chain shares only 50% identity with this octapeptide (Canine sequence: Pro-Leu-Asp-Leu-Tyr-Val-His-Lys). Based on this observation, attempts to use IgE peptides involved in Fcε receptor binding to modulate allergic reactions in dogs would require the use of peptides derived from the canine Igε sequence.

The sequences of the IgE constant regions from several species including human, rat and mouse have been reported. Peptides derived from known IgE sequences have been used to generate antibodies which alter IgE function. U. S. Patent 5,091,313 is directed to the prevention or control of IgE-mediated allergic symptoms through the use of monoclonal or polyclonal antibodies raised against epitopes present in B cell-associated or soluble human IgE. WO90/15878 discloses the use of peptides derived from human, rat or mouse IgE sequences to generate antibodies which inhibit IgE-mediated mast cell degranulation. U. S. Patent 4,223,016 discloses the use of peptides derived from IgE sequences for allergic desensitization.

The present invention identifies a species-specific sequence of the canine IgE constant region. For therapeutic purposes, it may be desirable to generate antibodies against the IgE of the target species in order to maximize the affinity of the anti-IgE antibodies. In addition, screening assays aimed at the identification of small molecules which alter IgE mediated responses in the dog can be optimized through the use of canine IgE, the actual target.

Prior to the described invention, it was virtually impossible to design peptides which could be used to produce antibodies of specifically targeted against canine IgE. When IgE sequences from other species are used for this purpose, the resulting antibodies have reduced affinity for the canine IgE and, therefore, reduced efficacy compared with antibodies generated using the described invention. Further, the availability of the cloned canine IgE gene enables large quantities of the canine IgE protein to be produced recombinantly for

use in drug development (e.g., small molecule screening, assay development and anti-IgE antibody generation).

The DNA of the present invention may be used to identify regions of the canine IgE which are homologous to those targeted in other species and to predict novel therapeutic targets. Therapeutically interesting portions of the sequence may be expressed in chimeric proteins or used to produce peptides. These molecules or conjugate derivatives thereof may then be used, with or without adjuvants, as canine vaccines to treat or prevent IgE mediated-hypersensitivity responses. Alternately, the derived peptides or proteins may be used to produce monoclonal or polyclonal antibodies for passive treatment of IgE-mediated hypersensitivity.

The invention also provides a renewable source of canine IgE protein through its expression using recombinant DNA techniques. This provides material for establishing assays to monitor IgE-mediated immune responses as well as for developing screens to identify small molecules capable of disrupting IgE-mediated allergic reactions in the dog.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the nucleotide and predicted amino acid sequences of canine immunoglobulin E.

Figure 2 shows a comparison of percent identity of nucleotide and amino acid sequence of canine IgE chain to human and mouse IgE chain.

Figure 3 shows the nucleotide and predicted amino acid sequences of canine immunoglobulin A.

Figure 4 shows a comparison of percent identity of nucleotide and amino acid sequence of canine Igα chain to human and mouse Igα chain.

SUMMARY OF THE INVENTION

The present invention relates to DNA molecules encoding a canine IgE and species-specific regions of the canine IgE

constant region. The invention comprises the DNA molecules, proteins encoded by the DNA molecules, antibodies to the proteins, cells transformed by the DNA molecules, assays employing the transformed cells, compounds identified by the assays and kits containing the DNA molecules or derivatives thereof.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to DNA molecules encoding a canine IgE and species-specific regions of the canine IgE constant region. The invention comprises the DNA molecules, proteins encoded by the DNA molecules, antibodies to the proteins, cells transformed by the DNA molecules, assays employing the transformed cells, compounds identified by the assays and kits containing the DNA molecules or derivatives thereof.

DNA encoding canine IgE from a particular species of canine may be used to isolate and purify homologues of canine IgE from other canines. To accomplish this, the first canine IgE DNA may be mixed with a sample containing DNA encoding homologues of canine IgE under appropriate hybridization conditions. The hybridized DNA complex may be isolated and the DNA encoding the homologous DNA may be purified therefrom.

It is known that there is a substantial amount of redundancy in the various codons which code for specific amino acids. Therefore, this invention is also directed to those DNA sequences which contain alternative codons which code for the eventual translation of the identical amino acid. For purposes of this specification, a sequence bearing one or more replaced codons will be defined as a degenerate variation. Also included within the scope of this invention are mutations either in the DNA sequence or the translated protein which do not substantially alter the ultimate physical properties of the expressed protein. For example, substitution of valine for leucine, arginine for lysine, or asparagine for glutamine may not cause a change in functionality of the polypeptide.

It is known that DNA sequences coding for a peptide may be altered so as to code for a peptide having properties that are different than those of the naturally-occurring peptide. Methods of altering the DNA sequences include, but are not limited to site directed mutagenesis.

As used herein, a "functional derivative" of canine IgE is a compound that possesses a biological activity (either functional or structural) that is substantially similar to the biological activity of canine IgE. The term "functional derivatives" is intended to include the "fragments," "variants," "degenerate variants," "analogs" and "homologues" or to "chemical derivatives" of canine IgE. The term "fragment" is meant to refer to any polypeptide subset of canine IgE. The term "variant" is meant to refer to a molecule substantially similar in structure and function to either the entire canine IgE molecule or to a fragment thereof. A molecule is "substantially similar" to canine IgE if both molecules have substantially similar structures or if both molecules possess similar biological activity. Therefore, if the two molecules possess substantially similar activity, they are considered to be variants even if the structure of one of the molecules is not found in the other or even if the two amino acid sequences are not identical. The term "analog" refers to a molecule substantially similar in function to either the entire canine IgE molecule or to a fragment thereof.

As used herein, a protein or peptide is "substantially pure" when that protein or peptide has been purified to the extent that it is essentially free of other molecules with which it is associated in nature. The term "substantially pure" is used relative to proteins or peptides with which the peptides of the instant invention are associated in nature, and are not intended to exclude compositions in which the peptide of the invention is admixed with nonproteinous pharmaceutical carriers or vehicles.

As used herein, an amino acid sequence substantially homologous to a referent IgE protein will have at least 70% sequence homology, preferably 80%, and most preferably 90% sequence

homology with the amino acid sequence of a referent IgE protein or a peptide thereof. For example, an amino acid sequence is substantially homologous to canine IgE protein if, when aligned with canine IgE protein, at least 70% of its amino acid residues are the same.

As used herein, a DNA sequence substantially homologous to a referent canine IgE protein will have at least 70%, preferably 80%, and most preferably 90% sequence homology with the DNA sequence of a referent canine IgE. Moreover, a DNA sequence substantially homologous to a referent canine IgE protein is characterized by the ability to hybridize to the DNA sequence of a referent canine IgE under standard conditions. Standard hybridization conditions are described in Maniatis, T., et al. (1989) Molecular Cloning, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

A variety of procedures known in the art may be used to molecularly clone canine IgE DNA. These methods include, but are not limited to, direct functional expression of the canine IgE genes following the construction of a canine IgE-containing cDNA or genomic DNA library in an appropriate expression vector system. Another method is to screen canine IgE-containing cDNA or genomic DNA library constructed in a bacteriophage or plasmid shuttle vector with a labeled oligonucleotide probe designed from the amino acid sequence of the canine IgE subunits. An additional method consists of screening a canine IgE-containing cDNA or genomic DNA libraries constructed in a bacteriophage or plasmid shuttle vector with a partial DNA encoding the canine IgE. This partial DNA is obtained by the specific PCR amplification of canine IgE DNA fragments through the design of degenerate oligonucleotide primers from the amino acid sequence of the purified canine IgE. Another method is to isolate RNA from canine IgE-producing cells and translate the RNA into protein via an *in vitro* or an *in vivo* translation system. The translation of the RNA into a peptide or a protein will result in the production of at least a portion of the canine IgE protein which can be identified by, for example, by the activity of canine IgE protein or by immunological

reactivity with an anti-canine IgE antibody. In this method, pools of RNA isolated from canine IgE-producing cells can be analyzed for the presence of an RNA which encodes at least a portion of the canine IgE protein. Further fractionation of the RNA pool can be done to purify the canine IgE RNA from non-canine IgE RNA. The peptide or protein produced by this method may be analyzed to provide amino acid sequences which in turn are used to provide primers for production of canine IgE cDNA, or the RNA used for translation can be analyzed to provide nucleotide sequences encoding canine IgE and produce probes for the production of canine IgE cDNA. These methods are known in the art and can be found in, for example, Sambrook, J., Fritsch, E.F., Maniatis, T. in Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. 1989.

Other types of libraries, as well as libraries constructed from other cells or cell types, may be useful for isolating canine IgE-encoding DNA. Other types of libraries include, but are not limited to, cDNA libraries derived from other canines or cell lines derived from other canines, and genomic DNA libraries.

Preparation of cDNA libraries can be performed by standard techniques. Well known cDNA library construction techniques can be found in, for example, Sambrook, J., et al., supra.

DNA encoding canine IgE may also be isolated from a suitable genomic DNA library. Construction of genomic DNA libraries can be performed by standard techniques. Well known genomic DNA library construction techniques can be found in Sambrook, J., et al, supra

In order to clone the canine IgE gene by the above methods, knowledge of the amino acid sequence of canine IgE may be necessary. To accomplish this, canine IgE protein may be purified and partial amino acid sequence determined by manual sequencing or automated sequenators. It is not necessary to determine the entire amino acid sequence, but the linear sequence of two regions of 6 to 8 amino acids from the protein is determined for

the production of primers for PCR amplification of a partial canine IgE DNA fragment.

Once suitable amino acid sequences have been identified, the DNA sequences capable of encoding them are synthesized. Because the genetic code is degenerate, more than one codon may be used to encode a particular amino acid, and therefore, the amino acid sequence can be encoded by any of a set of similar DNA oligonucleotides. Only one member of the set will be identical to the canine IgE sequence but will be capable of hybridizing to canine IgE DNA even in the presence of DNA oligonucleotides with mismatches under appropriate conditions. Under alternate conditions, the mismatched DNA oligonucleotides may still sufficiently hybridize to the canine IgE DNA to permit identification and isolation of canine IgE encoding DNA.

Purified biologically active canine IgE may have several different physical forms. Canine IgE may exist as a full-length nascent or unprocessed polypeptide, or as partially processed polypeptides or combinations of processed polypeptides. The full-length nascent canine IgE polypeptide may be postranslationally modified by specific proteolytic cleavage events which result in the formation of fragments of the full length nascent polypeptide.

Canine IgE in substantially pure form derived from natural sources or from recombinant host cells according to the purification processes described herein, is found to be a polypeptide encoded by a single mRNA

The cloned canine IgE DNA obtained through the methods described herein may be recombinantly expressed by molecular cloning into an expression vector containing a suitable promoter and other appropriate transcription regulatory elements, and transferred into prokaryotic or eukaryotic host cells to produce recombinant canine IgE. Techniques for such manipulations are fully described in Sambrook, J., et al., supra,.

Expression vectors are defined herein as DNA sequences that are required for the transcription of cloned copies of genes and

the translation of their mRNAs in an appropriate host. Such vectors can be used to express eukaryotic genes in a variety of hosts such as bacteria, bluegreen algae, plant cells, insect cells, fungal cells and animal cells.

Specifically designed vectors allow the shuttling of DNA between hosts such as bacteria-yeast or bacteria-animal cells or bacteria-fungal cells or bacteria-invertebrate cells. An appropriately constructed expression vector should contain: an origin of replication for autonomous replication in host cells, selectable markers, a limited number of useful restriction enzyme sites, a potential for high copy number, and active promoters. A promoter is defined as a DNA sequence that directs RNA polymerase to bind to DNA and initiate RNA synthesis. A strong promoter is one which causes mRNAs to be initiated at high frequency. Expression vectors may include, but are not limited to, cloning vectors, modified cloning vectors, specifically designed plasmids or viruses.

A variety of mammalian expression vectors may be used to express recombinant canine IgE in mammalian cells. Commercially available mammalian expression vectors which may be suitable for recombinant canine IgE expression, include but are not limited to, pcDNA3 (Invitrogen), pMC1neo (Stratagene), pXT1 (Stratagene), pSG5 (Stratagene), EBO-pSV2-neo (ATCC 37593) pBPV-1(8-2) (ATCC 37110), pdBPV-MMTneo(342-12) (ATCC 37224), pRSVgpt (ATCC 37199), pRSVneo (ATCC 37198), pSV2-dhfr (ATCC 37146), pUCTag (ATCC 37460), and λZD35 (ATCC 37565).

A variety of bacterial expression vectors may be used to express recombinant canine IgE in bacterial cells. Commercially available bacterial expression vectors which may be suitable for recombinant canine IgE expression include, but are not limited to pET11a (Novagen), lambda gt11 (Invitrogen), pcDNAII (Invitrogen), pKK223-3 (Pharmacia).

A variety of fungal cell expression vectors may be used to express recombinant canine IgE in fungal cells. Commercially

available fungal cell expression vectors which may be suitable for recombinant canine IgE expression include but are not limited to pYES2 (Invitrogen), *Pichia* expression vector (Invitrogen).

A variety of insect cell expression vectors may be used to express recombinant canine IgE in insect cells. Commercially available insect cell expression vectors which may be suitable for recombinant expression of canine IgE include but are not limited to pBlue Bac III (Invitrogen).

An expression vector containing DNA encoding canine IgE may be used for expression of canine IgE in a recombinant host cell. Recombinant host cells may be prokaryotic or eukaryotic, including but not limited to bacteria such as E. coli, fungal cells such as yeast, mammalian cells including but not limited to cell lines of human, bovine, porcine, monkey and rodent origin, and insect cells including but not limited to Drosophila and silkworm derived cell lines. Cell lines derived from mammalian species which may be suitable and which are commercially available, include but are not limited to, L cells L-M(TK$^-$) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C1271 (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) and MRC-5 (ATCC CCL 171).

The expression vector may be introduced into host cells via any one of a number of techniques including but not limited to transformation, transfection, lipofection, protoplast fusion, and electroporation. The expression vector-containing cells are clonally propagated and individually analyzed to determine whether they produce canine IgE protein. Identification of canine IgE expressing host cell clones may be done by several means, including but not limited to immunological reactivity with anti-canine IgE antibodies, and the presence of host cell-associated canine IgE activity, such as canine IgE-specific ligand binding or signal transduction defined as a

response mediated by the interaction of canine IgE-specific ligands at the receptor.

Expression of canine IgE DNA may also be performed using *in vitro* produced synthetic mRNA or native mRNA. Synthetic mRNA or mRNA isolated from canine IgE producing cells can be efficiently translated in various cell-free systems, including but not limited to wheat germ extracts and reticulocyte extracts, as well as efficiently translated in cell-based systems, including but not limited to microinjection into frog oocytes, with microinjection into frog oocytes being preferred.

Host cell transfectants and microinjected oocytes may be assayed for both the levels of canine IgE receptor activity and levels of canine IgE protein by a variety of methods.

Following expression of canine IgE in a recombinant host cell, canine IgE protein may be recovered to provide canine IgE in purified form. Several canine IgE purification procedures are available and suitable for use. As described herein, recombinant canine IgE may be purified from cell lysates and extracts by various combinations of, or individual application of salt fractionation, ion exchange chromatography, size exclusion chromatography, hydroxylapatite adsorption chromatography and hydrophobic interaction chromatography.

In addition, recombinant canine IgE can be separated from other cellular proteins by use of an immunoaffinity column made with monoclonal or polyclonal antibodies specific for full length nascent canine IgE, or polypeptide fragments of canine IgE.

Monospecific antibodies to canine IgE are purified from mammalian antisera containing antibodies reactive against canine IgE or are prepared as monoclonal antibodies reactive with canine IgE using the technique of Kohler and Milstein, *Nature* **256**, 495-497 (1975). Monospecific antibody as used herein is defined as a single antibody species or multiple antibody species with homogenous binding characteristics for canine IgE. Homogenous binding as used herein refers to the ability of the antibody species to bind to a

specific antigen or epitope, such as those associated with the canine IgE, as described above. Canine IgE specific antibodies are raised by immunizing animals such as mice, rats, guinea pigs, rabbits, goats, horses and the like, with rabbits being preferred, with an appropriate concentration of canine IgE either with or without an immune adjuvant.

Monoclonal antibodies (mAb) reactive with canine IgE are prepared by immunizing inbred mice, preferably Balb/c, with canine IgE. The mice are immunized by the IP or SC route with about 0.1 μg to about 10 μg, preferably about 1 μg, of canine IgE in about 0.5 ml buffer or saline incorporated in an equal volume of an acceptable adjuvant, as discussed above. Freund's complete adjuvant is preferred. The mice receive an initial immunization on day 0 and are rested for about 3 to about 30 weeks. Immunized mice are given one or more booster immunizations of about 0.1 to about 10 μg of canine IgE in a buffer solution such as phosphate buffered saline by the intravenous (IV) route. Lymphocytes, from antibody positive mice, preferably splenic lymphocytes, are obtained by removing spleens from immunized mice by standard procedures known in the art. Hybridoma cells are produced by mixing the splenic lymphocytes with an appropriate fusion partner, preferably myeloma cells, under conditions which will allow the formation of stable hybridomas. Fusion partners may include, but are not limited to: mouse myelomas P3/NS1/Ag 4-1; MPC-11; S-194 and Sp 2/0, with Sp 2/0 being preferred. The antibody producing cells and myeloma cells are fused in polyethylene glycol, about 1000 molecular weight, at concentrations from about 30% to about 50%. Fused hybridoma cells are selected by growth in hypoxanthine, thymidine and aminopterin supplemented Dulbecco's Modified Eagles Medium (DMEM) by procedures known in the art. Supernatant fluids are collected from growth positive wells on about days 14, 18, and 21 and are screened for antibody production by an immunoassay such as solid phase immunoradioassay (SPIRA) using canine IgE as the antigen. The culture fluids are also tested in the Ouchterlony

precipitation assay to determine the isotype of the mAb. Hybridoma cells from antibody positive wells are cloned by a technique such as the soft agar technique of MacPherson, Soft Agar Techniques, in *Tissue Culture Methods and Applications*, Kruse and Paterson, Eds., Academic Press, 1973.

Monoclonal antibodies are produced *in vivo* by injection of pristane primed Balb/c mice, approximately 0.5 ml per mouse, with about 2 x 10^6 to about 6 x 10^6 hybridoma cells about 4 days after priming. Ascites fluid is collected at approximately 8-12 days after cell transfer and the monoclonal antibodies are purified by techniques known in the art.

In vitro production of anti-canine IgE mAb is carried out by growing the hydridoma in DMEM containing about 2% fetal calf serum to obtain sufficient quantities of the specific mAb. The mAb are purified by techniques known in the art.

Antibody titers of ascites or hybridoma culture fluids are determined by various serological or immunological assays which include, but are not limited to, precipitation, passive agglutination, enzyme-linked immunosorbent antibody (ELISA) technique and radioimmunoassay (RIA) techniques. Similar assays are used to detect the presence of canine IgE in body fluids or tissue and cell extracts.

The above described methods for producing monospecific antibodies may be utilized to produce antibodies specific for canine IgE polypeptide fragments, or full-length nascent canine IgE polypeptide

The present invention is also directed to methods for screening for compounds which modulate the expression of DNA or RNA encoding canine IgE as well as the function of canine IgE protein *in vivo*. Compounds which modulate these activities may be DNA, RNA, peptides, proteins, or non-proteinaceous organic molecules. Compounds may modulate by increasing or attenuating the expression of DNA or RNA encoding canine IgE, or the function of canine IgE protein. Compounds that modulate the expression of

DNA or RNA encoding canine IgE or the function of canine IgE protein may be detected by a variety of assays. The assay may be a simple "yes/no" assay to determine whether there is a change in expression or function. The assay may be made quantitative by comparing the expression or function of a test sample with the levels of expression or function in a standard sample.

Kits containing canine IgE DNA, antibodies to canine IgE, or canine IgE protein may be prepared. Such kits are used to detect DNA which hybridizes to canine IgE DNA or to detect the presence of canine IgE protein or peptide fragments in a sample. Such characterization is useful for a variety of purposes including but not limited to forensic analyses and epidemiological studies.

The DNA molecules, RNA molecules, recombinant protein and antibodies of the present invention may be used to screen and measure levels of canine IgE DNA, canine IgE RNA or canine IgE protein. The recombinant proteins, DNA molecules, RNA molecules and antibodies lend themselves to the formulation of kits suitable for the detection and typing of canine IgE. Such a kit would comprise a compartmentalized carrier suitable to hold in close confinement at least one container. The carrier would further comprise reagents such as recombinant canine IgE protein or anti-canine IgE antibodies suitable for detecting canine IgE. The carrier may also contain a means for detection such as labeled antigen or enzyme substrates or the like.

Nucleotide sequences that are complementary to the canine IgE encoding DNA sequence can be synthesized for antisense therapy. These antisense molecules may be DNA, stable derivatives of DNA such as phosphorothioates or methylphosphonates, RNA, stable derivatives of RNA such as 2'-*O*-alkylRNA, or other canine IgE antisense oligonucleotide mimetics. canine IgE antisense molecules may be introduced into cells by microinjection, liposome encapsulation or by expression from vectors harboring the antisense sequence. canine IgE antisense therapy may be particularly useful

for the treatment of diseases where it is beneficial to reduce canine IgE activity.

Pharmaceutically useful compositions comprising canine IgE DNA, canine IgE RNA, or canine IgE protein, or modulators of canine IgE activity, may be formulated according to known methods such as by the admixture of a pharmaceutically acceptable carrier. Examples of such carriers and methods of formulation may be found in Remington's Pharmaceutical Sciences. To form a pharmaceutically acceptable composition suitable for effective administration, such compositions will contain an effective amount of the protein, DNA, RNA, or modulator.

Therapeutic or diagnostic compositions of the invention are administered to an individual in amounts sufficient to treat or diagnose canine IgE related disorders. The effective amount may vary according to a variety of factors such as the animal's condition, weight, sex and age. Other factors include the mode of administration.

The pharmaceutical compositions may be provided to the animal by a variety of routes such as subcutaneous, topical, oral and intramuscular.

The term "chemical derivative" describes a molecule that contains additional chemical moieties which are not normally a part of the base molecule. Such moieties may improve the solubility, half-life, absorption, etc. of the base molecule. Alternatively the moieties may attenuate undesirable side effects of the base molecule or decrease the toxicity of the base molecule. Examples of such moieties are described in a variety of texts, such as Remington's Pharmaceutical Sciences.

Compounds identified according to the methods disclosed herein may be used alone at appropriate dosages defined by routine testing in order to obtain optimal inhibition of the canine IgE or its activity while minimizing any potential toxicity. In addition, co-administration or sequential administration of other agents may be desirable.

The present invention also has the objective of providing suitable topical, oral, systemic and parenteral pharmaceutical formulations for use in the methods of treatment of the present invention. The compositions containing compounds identified according to this invention as the active ingredient for use in the modulation of canine IgE can be administered in a wide variety of therapeutic dosage forms in conventional vehicles for administration. For example, the compounds can be administered in such oral dosage forms as tablets, capsules (each including timed release and sustained release formulations), pills, powders, granules, elixirs, tinctures, solutions, suspensions, syrups and emulsions, or by injection. Likewise, they may also be administered in intravenous (both bolus and infusion), intraperitoneal, subcutaneous, topical with or without occlusion, or intramuscular form, all using forms well known to those of ordinary skill in the pharmaceutical arts. An effective but non-toxic amount of the compound desired can be employed as a canine IgE modulating agent.

The daily dosage of the products may be varied over a wide range. Advantageously, compounds of the present invention may be administered in a single daily dose, or the total daily dosage may be administered in divided doses of two, three or four times daily. Furthermore, compounds for the present invention can be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in that art. To be administered in the form of a transdermal delivery system, the dosage administration will, of course, be continuous rather than intermittent throughout the dosage regimen.

For combination treatment with more than one active agent, where the active agents are in separate dosage formulations, the active agents can be administered concurrently, or they each can be administered at separately staggered times.

The dosage regimen utilizing the compounds of the present invention is selected in accordance with a variety of factors

including type, species, age, weight, sex and medical condition of the animal, the severity of the condition to be treated, and the particular compound thereof employed. A veterinarian of ordinary skill can readily determine and prescribe the effective amount of the drug required to prevent, counter or arrest the progress of the condition. Optimal precision in achieving concentrations of drug within the range that yields efficacy without toxicity requires a regimen based on the kinetics of the drug's availability to target sites. This involves a consideration of the distribution, equilibrium, and elimination of a drug.

In the methods of the present invention, the compounds herein described in detail can form the active ingredient, and are typically administered in admixture with suitable pharmaceutical diluents, excipients or carriers (collectively referred to herein as "carrier" materials) suitably selected with respect to the intended form of administration, that is, oral tablets, capsules, elixirs, syrups and the like, and consistent with conventional pharmaceutical practices.

For instance, for oral administration in the form of a tablet or capsule, the active drug component can be combined with an oral, non-toxic pharmaceutically acceptable inert carrier such as ethanol, glycerol, water and the like. Moreover, when desired or necessary, suitable binders, lubricants, disintegrating agents and coloring agents can also be incorporated into the mixture. Suitable binders include, without limitation, starch, gelatin, natural sugars such as glucose or beta-lactose, corn sweeteners, natural and synthetic gums such as acacia, tragacanth or sodium alginate, carboxymethylcellulose, polyethylene glycol, waxes and the like. Lubricants used in these dosage forms include, without limitation, sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride and the like. Disintegrators include, without limitation, starch, methyl cellulose, agar, bentonite, xanthan gum and the like.

For liquid forms the active drug component can be combined in suitably flavored suspending or dispersing agents such as the synthetic and natural gums, for example, tragacanth, acacia, methylcellulose and the like. Other dispersing agents which may be employed include glycerin and the like. For parenteral administration, sterile suspensions and solutions are desired. Isotonic preparations which generally contain suitable preservatives are employed when intravenous administration is desired.

Topical preparations containing the active drug component can be admixed with a variety of carrier materials well known in the art, such as, e.g., alcohols, aloe vera gel, allantoin, glycerine, vitamin A and E oils, mineral oil, PPG2 myristyl propionate, and the like, to form, e.g., alcoholic solutions, topical cleansers, cleansing creams, skin gels, skin lotions, and shampoos in cream or gel formulations.

The compounds of the present invention can also be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, such as cholesterol, stearylamine or phosphatidylcholines.

Compounds of the present invention may also be delivered by the use of monoclonal antibodies as individual carriers to which the compound molecules are coupled. The compounds of the present invention may also be coupled with soluble polymers as targetable drug carriers. Such polymers can include polyvinylpyrrolidone, pyran copolymer, polyhydroxypropylmethacrylamidephenol, polyhydroxy-ethylaspartamidephenol, or polyethyleneoxidepolylysine substituted with palmitoyl residues. Furthermore, the compounds of the present invention may be coupled to a class of biodegradable polymers useful in achieving controlled release of a drug, for example, polylactic acid, polyepsilon caprolactone, polyhydroxy butyric acid, polyorthoesters, polyacetals, polydihydro-pyrans, polycyanoacrylates and cross-linked or amphipathic block copolymers of hydrogels.

The following examples illustrate the present invention without, however, limiting the same thereto.

EXAMPLE 1

Genomic Cloning

A canine liver genomic DNA bactreriophage library was purchased from Clontech Inc. and 1 x10^6 individual plaques were screened with a 4.3 kb XhoI-EcoRI fragment containing the entire human IgA constant region gene (Kirsch et. al.) essentially as described in Hieter, P., et al.,1981, Nature. 294: 536-540 and Gazdar, A., et al., 1986, Blood. 67:1542-1549. Filters were hybridized overnight at 42°C in a 10% Dextran Sulfate, 4x SSC, 50% formamide, 0.8% Denhardt's Tris buffered solution. After hybridization, filters were washed with 2x SSC, 0.1% SDS at room temperature for 30 minutes, 1x SSC, 0.1% SDS at room temperature for 30 minutes and 1x SSC, 0.1% SDS at 42°C for 30 minutes. Five positive bacteriophage were plaque purified, and large scale lysates were prepared. Restriction mapping of positive bacteriophage clones were performed according to manufacturer's suggested conditions with the restriction enzymes indicated. Regions of the clones containing the canine IgA and IgE constant region were identified using the human IgA constant region probe described above and a 2.8 kb BamHI fragment encoding the human genomic IgE constant region (Kirsch et. al.). One clone, clone 19, contained two SstI fragments, 1.2 and 1.9 kb that hybridized to the human IgE constant region probe. These fragments were excised and clone into the SstI site of Bluescript (Stratagene).

EXAMPLE 2

Nucleotide Sequence Analysis

The DNA sequence of relevant regions of the canine IgE constant region genes was determined by the "dideoxy" chain termination method using the USB Sequenase DNA sequencing kit. Synthetic oligonucleotides used as sequencing primers were synthesized on an ABI 381 synthesizer or purchased from Stratagene. Nucleic acid alignments ad translations were done using the University of Wisconsin Sequence analysis software package (Devereux, J., P. Haeverli, and O. Smithies. 1984. Nuc. Acid. Res. 12: 387-395).

EXAMPLE 3

Genomic DNA Extraction and Analysis

Genomic DNA was prepared (Basic Methods in Molecular Biology Eds. Davis, L., Dibner, M., and Battey, J. Elsevier New York 1986) from canine liver or purchased from Clontech. 10 µg of canine liver genomic DNA was digested to completion with the restriction enzymes BamHI, EcoRI, XbaI and SalI (BMB) as specified by the supplier, fractionated on a 0.8% agarose gel, and transferred to nitrocellulose paper by the method of Southern. Canine IgE constant region gene probes were labelled with [^{32}P] by nick translation and consisted of a) 1.2 kb SstI fragment containing the CH_1 and part of the CH_2 coding region, b) 300 b.p. ApaI-SstI fragment containing part of the CH_2 coding region, and c) a 180 b.p. XhoI-BamHI fragment containing part of the CH_1 coding region.

Initial genomic Southern blot analyis using both human and mouse IgE constant region probes failed to detect canine IgE constant region sequences under reduced stringency blot washing conditions. Previous work showed that IgA constant region genes are more closely conserved from species to species than IgE constant region genes, but are closely linked to the IgE sequences. Therefore, a DNA fragment containing the human IgA constant region gene was used as a probe to screen a canine genomic liver DNA bacteriophage library to isolate recombinant clones containing the canine IgA constant region gene. Five positive bacteriophage clones were identified and plaque purified. Each of these clones was probed with the human IgA and IgE constant region gene fragments and one of the clones, clone 19, was shown to have sequences that hybridized to both the human IgA and IgE constant region gene probes. This clone was further characterized.

Initial restriction mapping and Southern blot analysis suggested that the canine IgE constant region gene was encoded on two SstI fragments 1.2 and 1.9 kb in size. These restriction fragments were subcloned and detailed nucleotide sequence analyis was performed. This sequence analysis demonstrated that these two fragments contained the

entire coding region of the canine IgE constant region gene and that the common SstI restriction enzyme site shared by the two fragments was contained within the CH2 coding sequence. The entire canine IgE constant region gene is encoded in four exons spread out over 2 kb.

EXAMPLE 4

Cloning Of Of Canine IgE For Expression Of The Canine IgE Polypeptide In Other Host Cell Systems

a) Cloning of Canine IgE cDNA into a bacterial expression vector. Recombinant Canine IgE is produced in a bacterium such as *E.coli* following the insertion of the optimal canine IgE cDNA sequence into expression vectors designed to direct the expression of heterologous proteins. These vectors are constructed such that recombinant canine IgE is synthesized alone or as a fusion protein for subsequent manipulation. Expression may be controlled such that recombinant canine IgE is recovered as a soluble protein or within insoluble inclusion bodies. Vectors such as pBR322, pSKF, pUR, pATH, pGEX, pT7-5, pT7-6, pT7-7, pET, pIBI (IBI), pSP6/T7-19 (Gibco/BRL), pBluescript II (Stratagene), pTZ18R, pTZ19R (USB), pSE420 (Invitrogen) or the like are suitable for these purposes.

b) Cloning of Canine IgE cDNA into a yeast expression vector Recombinant Canine IgE is produced in a yeast such as *Saccharomyces cerevisiae* following the insertion of the optimal canine IgE cDNA cistron into expression vectors designed to direct the intracellular or extracellular expression of heterologous proteins. In the case of intracellular expression, vectors such as EmBLyex4 or the like are ligated to the canine IgE cistron (Rinas, U. et al., Biotechnology 8: 543-545 (1990); Horowitz B. et al., J. Biol. Chem. 265: 4189-4192 (1989)). For extracellular expression, the canine IgE cistron is ligated into yeast expression vectors which fuse a secretion signal (a yeast or mammalian peptide) to the amino terminus of the canine IgE protein (Jacobson, M. A., Gene 85: 511-516 (1989); Riett L. and Bellon N. Biochem. 28: 2941-2949 (1989)).

c) Cloning of Canine IgE cDNA into a viral expression vector Recombinant canine IgE is produced in mammalian host cells, such as HeLa S3 cells, after infection with vaccinia virus containing the canine IgE cDNA sequence. To produce canine IgE:vaccinia virus, the canine IgE cDNA is first ligated into a transfer vector, such as pSC11, pTKgptF1s, pMJ601 or other suitable vector, then transferred to vaccinia virus by homologous recombination. After plaque purification and virus amplification, canine IgE:vaccinia virus is used to infect mammalian host cells and produce recombinant canine IgE protein.

EXAMPLE 5

Process for the production of a recombinant canine IgE polypeptide

Recombinant canine IgE is produced by

a) transforming a host cell with DNA encoding canine IgE protein to produce a recombinant host cell;

b) culturing the recombinant host cell under conditions which allow the production of canine IgE; and

c) recovering the canine IgE.

The recombinant canine IgE is purified and characterized by standard methods.

EXAMPLE 6

Compounds that modulate canine IgE activity may be detected by a variety of methods. A method of identifying compounds that affect canine IgE comprises:

(a) mixing a test compound with a solution containing canine IgE to form a mixture;

(b) measuring canine IgE activity in the mixture; and

(c) comparing the canine IgE activity of the mixture to a standard.

Compounds that modulate canine IgE activity may be formulated into pharmaceutical compositions. Such pharmaceutical

compositions may be useful for treating diseases or conditions that are characterized by altered canine IgE activity. Examples of such diseases wherein the canine IgE activity is altered include allergic reactions.

EXAMPLE 7

DNA which is structurally related to DNA encoding canine IgE is detected with a probe. A suitable probe may be derived from DNA having all or a portion of the nucleotide sequence of Figure 1, RNA encoded by DNA having all or a portion of the nucleotide sequence of Figure 1, degenerate oligonucleotides derived from a portion of the amino acid sequence of Figure 1 or an antibody directed against canine IgE.

EXAMPLE 8

A kit for the detection and characterization of DNA or RNA encoding canine IgE or canine IgE is prepared by conventional methods. The kit may contain DNA encoding canine IgE, recombinant canine IgE, RNA corresponding to the DNA encoding canine IgE or antibodies to canine IgE. The kit may be used to characterize test samples, such as forensic samples, taxonomic samples or epidemiological samples.

EXAMPLE 9

Use of mutagenized Canine IgE

DNA encoding Canine IgE is mutagenized using standard methods to produce an altered Canine IgE gene. Host cells are transformed with the altered Canine IgE to produce altered Canine IgE protein. The altered Canine IgE protein may be isolated, purified and used to characterize the function of Canine IgE protein.

EXAMPLE 10

Preparation of Immunogenic Compositions

Purified recombinant canine IgE are formulated according to known methods, such as by the admixture of a pharmaceutically acceptable carrier or a vaccine adjuvant. The amount of canine IgE per formulation may vary according to a variety of factors, including but not limited to the animal's condition, weight, age and sex. Such formulations are administered to an animal in amounts sufficient to induce an immune response in the animal. Administration of the recombinant canine IgE formulation may be by a variety of routes, including but not limited to oral, subcutaneous, topical, mucosal and intramuscular.

EXAMPLE 11

Preparation of Antibodies to canine IgE

Purified recombinant canine IgE is used to generate antibodies. The term "antibody" as used herein includes both polyclonal and monoclonal antibodies as well as fragments thereof, such as Fv, Fab and F(ab)2 fragments that are capable of binding antigen or hapten. The antibodies are used in a variety of ways, including but not limited to the purification of recombinant canine IgE , the purification of native canine IgE, and kits. Kits would comprise a compartmentalized carrier suitable to hold in close confinement at least one container. The carrier would further comprise reagents such as the anti-canine IgE antibody or the recombinant canine IgE suitable for detecting canine IgE or fragments of canine IgE or antibodies to canine IgE. The carrier may also contain means for detection such as labeled antigen or enzyme substrates or the like. The antibodies or canine IgE or kits are useful for a variety of purposes, including but not limited to forensic analyses and epidemiological studies.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: HOLLIS, GREGORY F.
PATEL, MAYUR D.

(ii) TITLE OF INVENTION: DNA ENCODING CANINE IMMUNOGLOBULINS

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: CHRISTINE E. CARTY
(B) STREET: 126 E. LINCOLN AVENUE; P.O. BOX 2000
(C) CITY: RAHWAY
(D) STATE: NEW JERSEY
(E) COUNTRY: USA
(F) ZIP: 07065-0907

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/336,586
(B) FILING DATE: 09-NOV-1994
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: CARTY, CHRISTINE E.
(B) REGISTRATION NUMBER: 36,099
(C) REFERENCE/DOCKET NUMBER: 19211Y

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (908) 594-6734
(B) TELEFAX: (908) 594-4720

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1927 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CAGAGCAGAT ACCCAGGTCA ACAGCGGGCC TGGCATATGA TGGGGTGACA GTCCCCAAGG 60

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TGGCCTCCTG CTGTAAAGAC AACATCGCCA GTACCTCTGT TACACTGGGC TGTCTGGTCA 180

CCGGCTATCT CCCCATGTCG ACAACTGTGA CCTGGGACAC GGGGTCTCTA AATAAGAATG 240

TCACGACCTT CCCCACCACC TTCCACGAGA CCTACGGCCT CCACAGCATC GTCAGCCAGG 300

TGACCGCCTC GGGCAAGTGG GCCAAACAGA GGTTCACCTG CAGCGTGGCT CACGCTGAGT 360

CCACCGCCAT CAACAAGACC TTCAGTGGTA AGCCAGGGTT GGGCTGGCCC ACATGACACT 420

GGAGGGAGAA GGGACAGGCT GGGCGGGAGT GGTAGGAGAG GGGTGGTGGG CGGGCCCGAT 480

GCCGCCATGG CTGGTAACGC CCAGCACATG TGGGGCTGGG GCTGACACAT GAGTCCCGTG 540

GGCTCAGAGA CACCACTGCC ACATGGCTGC CTCTACTTCT AGCATGTGCC TTAAACTTCA 600

TTCCGCCTAC CGTGAAGCTC TTCCACTCCT CCTGCAACCC CGTCGGTGAT ACCCACACCA 660

CCATCCAGCT CCTGTGCCTC ATCTCTGGCT ACGTCCCAGG TGACATGGAG GTCATCTGGC 720

TGGTGGATGG GCAAAAGGCT ACAAACATAT TCCCATACAC TGCACCCGGC ACAAAGGAGG 780

GCAACGTGAC CTCTACCCAC AGCGAGCTCA ACATCACCCA GGGCGAGTGG GTATCCCAAA 840

AAACCTACAC CTGCCAGGTC ACCTATCAAG GCTTTACCTT TAAAGATGAG GCTCGCAAGT 900

GCTCAGGTAT GGCCCCCCTG TCCCCCAGAA ACCCAGATGC GCGAGGCTCA GAGATGAGGG 960

CCAAGGCACG CCCTCATGCA GCCTCTCACA CACTGCAGAG TCCGACCCCC GAGGCGTGAC 1020

GAGCTACCTG AGCCCACCCA GCCCCCTTGA CCTGTATGTC CACAAGGCGC CCAAGATCAC 1080

CTGCCTGGTA GTGGACCTGG CCACCATGGA AGGCATGAAC CTGACCTGGT ACCGGGAGAG 1140

CAAAGAACCC GTGAACCCGG GCCCTTTGAA CAAGAAGGAT CACTTCAATG GGACGATCAC 1200

AGTCACGTCT ACCCTGCCAG TGAACACCAA TGACTGGATC GAGGGCGAGA CCTACTATTG 1260

CAGGGTGACC CACCCGCACC TGCCCAAGGA CATCGTGCGC TCCATTGCCA AGGCCCCTGG 1320

TGAGCCACGG GCCCAGGGGA GGTGGGCGGG CCTCCTGAGC CGGAGCCTGG GCTGACCCCA 1380

CACCTATCCA CAGGCAAGCG TGCCCCCCCG GATGTGTACT TGTTCCTGCC ACCGGAGGAG 1440

GAGCAGGGGA CCAAGGACAG AGTCACCCTC ACGTGCCTGA TCCAGAACTT CTTCCCCGCG 1500

GACATTTCAG TGCAATGGCT GCGAAACGAC AGCCCCATCC AGACAGACCA GTACACCACC 1560

ACGGGGCCCC ACAAGGTCTC GGGCTCCAGG CCTGCCTTCT TCATCTTCAG CCGCCTGGAG 1620

GTTAGCCGGG TGGACTGGGA GCAGAAAAAC AAATTCACCT GCCAAGTGGT GCATGAGGCG 1680

CTGTCCGGCT CTAGGATCCT CCAGAAATGG GTGTCCAAAA CCCCCGGTAA ATGATGCCCA 1740

CCCTCCTCCC GCCGCCACCC CCCAGGGCTC CACCTGCTGG GAGGGAGGGG GGCTGGCAAG 1800

ACCCTCCATC TGTCCTTGTC AATAAACACT CCAGTGTCTG CTTGGAGCCC TGGGCACACC 1860

CATTTCTTGG GGGTGGGCAG GGTTGCAGAG CAGGGATGTC TTGGCACAGA AGGGTCCCCC 1920

AGGGTGT 1927

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 426 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Thr Ser Gln Asp Leu Ser Val Phe Pro Leu Ala Ser Cys Cys Lys Asp
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            20                  25                  30

Leu Pro Met Ser Thr Thr Val Thr Trp Asp Thr Gly Ser Leu Asn Lys
        35                  40                  45

Asn Val Thr Thr Phe Pro Thr Thr Phe His Glu Thr Tyr Gly Leu His
    50                  55                  60

Ser Ile Val Ser Gln Val Thr Ala Ser Gly Lys Trp Ala Lys Gln Arg
65                  70                  75                  80

Phe Thr Cys Ser Val Ala His Ala Glu Ser Thr Ala Ile Asn Lys Thr
                85                  90                  95

Phe Ser Ala Cys Ala Leu Asn Phe Ile Pro Pro Thr Val Lys Leu Phe
            100                 105                 110
His Ser Ser Cys Asn Pro Val Gly Asp Thr His Thr Thr Ile Gln Leu
        115                 120                 125

Leu Cys Leu Ile Ser Gly Tyr Val Pro Gly Asp Met Glu Val Ile Trp
    130                 135                 140

Leu Val Asp Gly Gln Lys Ala Thr Asn Ile Phe Pro Tyr Thr Ala Pro
145                 150                 155                 160

Gly Thr Lys Glu Gly Asn Val Thr Ser Thr His Ser Glu Leu Asn Ile
                165                 170                 175
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Thr Gln Gly Glu Trp Val Ser Gln Lys Thr Tyr Thr Cys Gln Val Thr
180 185 190

Tyr Gln Gly Phe Thr Phe Lys Asp Glu Ala Arg Lys Cys Ser Glu Ser
195 200 205

Asp Pro Arg Gly Val Thr Ser Tyr Leu Ser Pro Pro Ser Pro Leu Asp
210 215 220

Leu Tyr Val His Lys Ala Pro Lys Ile Thr Cys Leu Val Val Asp Leu
225 230 235 240

Ala Thr Met Glu Gly Met Asn Leu Thr Trp Tyr Arg Glu Ser Lys Glu
245 250 255

Pro Val Asn Pro Gly Pro Leu Asn Lys Lys Asp His Phe Asn Gly Thr
260 265 270

Ile Thr Val Thr Ser Thr Leu Pro Val Asn Thr Asn Asp Trp Ile Glu
275 280 285

Gly Glu Thr Tyr Tyr Cys Arg Val Thr His Pro His Leu Pro Lys Asp
290 295 300

Ile Val Arg Ser Ile Ala Lys Ala Pro Gly Lys Arg Ala Pro Pro Asp
305 310 315 320

Val Tyr Leu Phe Leu Pro Pro Glu Glu Glu Gln Gly Thr Lys Asp Arg
325 330 335

Val Thr Leu Thr Cys Leu Ile Gln Asn Phe Phe Pro Ala Asp Ile Ser
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Val Gln Trp Leu Arg Asn Asp Ser Pro Ile Gln Thr Asp Gln Tyr Thr
355 360 365

Thr Thr Gly Pro His Lys Val Ser Gly Ser Arg Pro Ala Phe Phe Ile
370 375 380

Phe Ser Arg Leu Glu Val Ser Arg Val Asp Trp Glu Gln Lys Asn Lys
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Phe Thr Cys Gln Val Val His Glu Ala Leu Ser Gly Ser Arg Ile Leu
405 410 415

Gln Lys Trp Val Ser Lys Thr Pro Gly Lys
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1789 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AGTGACCTAG CGTGTCATTC TGACCCAGGT CTCGGCATAT GAACTGCATG ACCTTGGGCT 60

GTCACTGACC ATCTCTATGC AGTTTCCTCT AGTGCAAAGA AAAAATAGCC CTCACCCTGC 120

CTGTGAGGCC ATGTAAGGGG TCCAGACAGC ACTGGCCCAC CAGCTCACAG AGTGTCCTGT 180

GTCACAGAGT CCAAAACCAG CCCCAGTGTG TTCCCGCTGA GCCTCTGCCA CCAGGAGTCA 240

GAAGGGTACG TGGTCATCGG CTGCCTGGTG CAGGGATTCT TCCCACCGGA GCCTGTGAAC 300

GTGACCTGGA ATGCCGGCAA GGACAGCACA TCTGTCAAGA ACTTCCCCCC CATGAAGGCT 360

GCTACCGGAA GCCTATACAC CATGAGCAGC CAGTTGACCC TGCCAGCCGC CCAGTGCCCT 420

GATGACTCGT CTGTGAAATG CCAAGTGCAG CATGCTTCCA GCCCCAGCAA GGCAGTGTCT 480

GTGCCCTGCA AAGGTCAGAG GGCAGGCTGG GGAGGGGCAG GGGCCCCACA TCCTCACTCT 540

GACCCTCCAC TTGGAGTTCT GGCCCCAAGG ACACTCCACG GGGAGGACAG TGGGCTGCTG 600

GGCTGAGCTC CCAGCAAGTG GCCAAGGTGG GGCCTCCATG AAGGACCTGG AGGGTGGCAG 660

GGGGCAGGCA GGCAGAGGGT GCACACTGAC CTGTTCCAAT CTCTCTCTCT CTCTCTCTCT 720

CTCTCTCTGC TCCTGAAGAT AACAGTCATC CGTGTCATCC ATGTCCCTCG TGCAATGAGC 780

CCCGCCTGTC ACTACAGAAG CCAGCCCTCG AGGATCTGCT TTTAGGCTCC AATGCCAGCC 840

TCACATGCAC ACTGAGTGGC CTGAAAGACC CCAAGGGTGC CACCTTCACC TGGAACCCCT 900

CCAAAGGGAA GGAACCCATC CAGAAGAATC CTGAGCGTGA CTCCTGTGGC TGCTACAGTG 960

TGTCCAGTGT CCTACCAGGC TGTGCTGATC CATGGAACCA TGGGGACACC TTCTCCTGCA 1020

CAGCCACCCA CCCTGAATCC AAGAGCCCGA TCACTGTCAG CATCACCAAA ACCACAGGTG 1080

GGCCCAGACC CTGCCCGTGA GGCACTGCTT GGCACACAAA AGTTTGTGAG GCAACTCCTA 1140

AGCCTGCTTC CTTCCTCTAG CCCCTGGGCT TGGGTGCTCC CACCCACATT TTACAAAGGG 1200

AAACTGTGGC ATGGGGTGCT ATGGGGAAGA AGGCTCTTCC CCCACCCCAG ATCCCTGACC 1260

TGGCTCTCTG TCCTGCAGAG CACATCCCGC CCCAGGTCCA CCTGCTGCCG CCGCCGTCGG 1320

AAGAGCTGGC CCTCAATGAG CTGGTGACAC TGACGTGCTT GGTGAGGGGC TTCAAACCAA 1380

AAGATGTGCT CGTACGATGG CTGCAAGGGA CCCAGGAGCT ACCCCAAGAG AAGTACTTGA 1440

CCTGGGAGCC CCTGAAGGAG CCTGACCAGA CCAACATGTT TGCCGTGACC AGCATGCTGA 1500

GGGTGACAGC CGAAGACTGG AAGCAGGGGG AGAAGTTCTC CTGCATGGTG GGCCACGAGG 1560

CTCTGCCCAT GTCCTTCACC CAGAAGACCA TCGACCGCCT GGCGGGTAAA CCCACCCACG 1620

TCAACGTGTC TGTGGTCATG GCAGAGGTGG ACGGCATCTG CTACTAAACC GCCCAATCTT 1680

CCCTCCCTAA ATAAACTCCA TGCTTGCCCA AAGCAGCCCC GTGCTTCCAT CAGGCCGCCT 1740

GTCTGTCCAT ATTCGGGGTC TGTGGCATAC TGAGGCAGGG GTAGAGCTC 1789

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 343 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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Ser Glu Gly Tyr Val Val Ile Gly Cys Leu Val Gln Gly Phe Phe Pro
            20                  25                  30

Pro Glu Pro Val Asn Val Thr Trp Asn Ala Gly Lys Asp Ser Thr Ser
        35                  40                  45

Val Lys Asn Phe Pro Pro Met Lys Ala Ala Thr Gly Ser Leu Tyr Thr
    50                  55                  60

Met Ser Ser Gln Leu Thr Leu Pro Ala Ala Gln Cys Pro Asp Asp Ser
65                  70                  75                  80

Ser Val Lys Cys Gln Val Gln His Ala Ser Ser Pro Ser Lys Ala Val
                85                  90                  95

Ser Val Pro Cys Lys Asp Asn Ser His Pro Cys His Pro Cys Pro Ser
            100                 105                 110

Cys Asn Glu Pro Arg Leu Ser Leu Gln Lys Pro Ala Leu Glu Asp Leu
        115                 120                 125

Leu Leu Gly Ser Asn Ala Ser Leu Thr Cys Thr Leu Ser Gly Leu Lys
    130                 135                 140
```

Asp Pro Lys Gly Ala Thr Phe Thr Trp Asn Pro Ser Lys Gly Lys Glu
145 150 155 160

Pro Ile Gln Lys Asn Pro Glu Arg Asp Ser Cys Gly Cys Tyr Ser Val
165 170 175

Ser Ser Val Leu Pro Gly Cys Ala Asp Pro Trp Asn His Gly Asp Thr
180 185 190

Phe Ser Cys Thr Ala Thr His Pro Glu Ser Lys Ser Pro Ile Thr Val
195 200 205

Ser Ile Thr Lys Thr Thr Glu His Ile Pro Pro Gln Val His Leu Leu
210 215 220

Pro Pro Pro Ser Glu Glu Leu Ala Leu Asn Glu Leu Val Thr Leu Thr
225 230 235 240

Cys Leu Val Arg Gly Phe Lys Pro Lys Asp Val Leu Val Arg Trp Leu
245 250 255

Gln Gly Thr Gln Glu Leu Pro Gln Glu Lys Tyr Leu Thr Trp Glu Pro
260 265 270

Leu Lys Glu Pro Asp Gln Thr Asn Met Phe Ala Val Thr Ser Met Leu
275 280 285

Arg Val Thr Ala Glu Asp Trp Lys Gln Gly Glu Lys Phe Ser Cys Met
290 295 300

Val Gly His Glu Ala Leu Pro Met Ser Phe Thr Gln Lys Thr Ile Asp
305 310 315 320

Arg Leu Ala Gly Lys Pro Thr His Val Asn Val Ser Val Val Met Ala
325 330 335

Glu Val Asp Gly Ile Cys Tyr
340

WHAT IS CLAIMED IS:

1. An isolated and purified DNA molecule which encodes canine immunoglobulin E or a functional derivative thereof.

2. The isolated and purified DNA molecule of claim 1, having a nucleotide sequence of Figure 1 (SEQ.ID.NO.:1) or a functional derivative thereof.

3. The isolated and purified DNA molecule of Claim 1, wherein the DNA molecule is genomic DNA.

4. An expression vector for expression of canine immunoglobulin E in a recombinant host, wherein the vector contains a recombinant gene encoding canine immunoglobulin E or functional derivative thereof.

5. The expression vector of claim 4, wherein the expression vector contains a cloned gene encoding canine immunoglobulin E, having a nucleotide sequence of Figure 1 (SEQ.ID.NO.:1) or a functional derivative thereof.

6. The expression vector of claim 4, wherein the expression vector contains genomic DNA encoding canine immunoglobulin E.

7. A recombinant host cell containing a recombinantly cloned gene encoding canine immunoglobulin E or functional derivative thereof.

8. The recombinant host cell of claim 7, wherein the gene encoding the canine immunoglobulin E has a nucleotide sequence of Figure 1 (SEQ.ID.NO.:1) or functional derivative thereof.

9. The recombinant host cell of claim 7, wherein the cloned gene encoding the canine immunoglobulin E is genomic DNA.

10. A protein, in substantially pure form which functions as canine immunoglobulin E.

11. The protein according to claim 10, having an amino acid sequence of Figure 1 (SEQ.ID.NO.:2) or a functional derivative thereof.

12. A monospecific antibody immunologically reactive with canine immunoglobulin E.

13. The antibody of Claim 12, wherein the antibody blocks activity of canine immunoglobulin E.

14. A process for expression of canine immunoglobulin E protein in a recombinant host cell, comprising:

(a) transferring the expression vector of Claim 4 into suitable host cells; and

(b) culturing the host cells of step (a) under conditions which allow expression of the canine immunoglobulin E protein from the expression vector.

15. A method of identifying compounds that modulate canine immunoglobulin E activity, comprising:

(a) combining a suspected modulator of canine immunoglobulin E activity with canine immunoglobulin E; and

(b) measuring an effect of the modulator on the canine immunoglobulin E activity.

16. A compound active in the method of Claim 15, wherein the compound is a modulator of canine immunoglobulin E.

17. A pharmaceutical composition comprising a compound active in the method of Claim 15, wherein the compound is a modulator of canine immunoglobulin E activity.

18. A method of treating an animal in need of such treatment for a condition which is mediated by canine immunoglobulin E activity, comprising administration of a canine immunoglobulin E modulating compound active in the method of Claim 15.

19. An isolated and purified DNA molecule which encodes canine immunoglobulin A or a functional derivative thereof.

20. The isolated and purified DNA molecule of claim 1, having a nucleotide sequence of Figure 1 or a functional derivative thereof.

```
                10                  30                  50
                .                   .                   .
1   CAGAGCAGATACCCAGGTCAACAGCGGGCCTGGCATATGATGGGGTGACAGTCCCCAAGG  60

                70                  90                  110
                .                   .                   .
61  CAGGCACTGACACTGGCCCTGTCCCCACAGCCACCAGCCAGGACCTGTCTGTGTTCCCCT  120
                                          XXThrSerGlnAspLeuSerValPheProL

                130                 150                 170
                .                   .                   .
121 TGGCCTCCTGCTGTAAAGACAACATCGCCAGTACCTCTGTTACACTGGGCTGTCTGGTCA  180
    euAlaSerCysCysLysAspAsnIleAlaSerThrSerValThrLeuGlyCysLeuValT

                190                 210                 230
                .                   .                   .
181 CCGGCTATCTCCCCATGTCGACAACTGTGACCTGGGACACGGGGTCTCTAAATAAGAATG  240
    hrGlyTyrLeuProMetSerThrThrValThrTrpAspThrGlySerLeuAsnLysAsnV

                250                 270                 290
                .                   .                   .
241 TCACGACCTTCCCCACCACCTTCCACGAGACCTACGGCCTCCACAGCATCGTCAGCCAGG  300
    alThrThrPheProThrThrPheHisGluThrTyrGlyLeuHisSerIleValSerGlnV

                310                 330                 350
                .                   .                   .
301 TGACCGCCTCGGGCAAGTGGGCCAAACAGAGGTTCACCTGCAGCGTGGCTCACGCTGAGT  360
    alThrAlaSerGlyLysTrpAlaLysGlnArgPheThrCysSerValAlaHisAlaGluS

                370                 390                 410
                .                   .                   .
361 CCACCGCCATCAACAAGACCTTCAGTGGTAAGCCAGGGTTGGGCTGGCCCACATGACACT  420
    erThrAlaIleAsnLysThrPheSerA

                430                 450                 470
                .                   .                   .
421 GGAGGGAGAAGGGACAGGCTGGGCGGGAGTGGTAGGAGAGGGGTGGTGGGCGGGCCCGAA  480

                490                 510                 530
                .                   .                   .
481 TGCCGCCATGGCTGGTAACGCCCAGCACATGTGGGGCTGGGGCTGACACATGAGTCCCGT  540
```

FIG. 1A

```
          550                 570                 590
541 GGGCTCAGAGACACCACTGCCACATGGCTGCCTCTACTTCTAGCATGTGCCTTAAACTTC 600
                                                laCysAlaLeuAsnPhe

          610                 630                 650
601 ATTCCGCCTACCGTGAAGCTCTTCCACTCCTCCTGCAACCCCGTCGGTGATACCCACACC 660
    IleProProThrValLysLeuPheHisSerSerCysAsnProValGlyAspThrHisThr

          670                 690                 710
661 ACCATCCAGCTCCTGTGCCTCATCTCTGGCTACGTCCCAGGTGACATGGAGGTCATCTGG 720
    ThrIleGlnLeuLeuCysLeuIleSerGlyTyrValProGlyAspMetGluValIleTrp

          730                 750                 770
721 CTGGTGGATGGGCAAAAGGCTACAAACATATTCCCATACACTGCACCCGGCACAAAGGAG 780
    LeuValAspGlyGlnLysAlaThrAsnIlePheProTyrThrAlaProGlyThrLysGlu

          790                 810                 830
781 GGCAACGTGACCTCTACCCACAGCGAGCTCAACATCACCCAGGGCGAGTGGGTATCCCAA 840
    GlyAsnValThrSerThrHisSerGluLeuAsnIleThrGlnGlyGluTrpValSerGln

          850                 870                 890
841 AAAACCTACACCTGCCAGGTCACCTATCAAGGCTTTACCTTTAAAGATGAGGCTCGCAAG 900
    LysThrTyrThrCysGlnValThrTyrGlnGlyPheThrPheLysAspGluAlaArgLys

          910                 930                 950
901 TGCTCAGGTATGGCCCCCCTGTCCCCCAGAAACCCAGATGCGCGAGGCTCAGAGATGAGG 960
    CysSerG

          970                 990                 1010
961 GCCAAGGCACGCCCTCATGCAGCCTCTCACACACTGCAGAGTCCGACCCCCGAGGCGTGA 1020
                                                luSerAspProArgGlyValT
```

FIG 1B

```
          1030                1050                1070
1021 CGAGCTACCTGAGCCCACCCAGCCCCCTTGACCTGTATGTCCACAAGGCGCCCAAGATCA 1080
     hrSerTyrLeuSerProProSerProLeuAspLeuTyrValHisLysAlaProLysIleT

          1090                1110                1130
1081 CCTGCCTGGTAGTGGACCTGGCCACCATGGAAGGCATGAACCTGACCTGGTACCGGGAGA 1140
     hrCysLeuValValAspLeuAlaThrMetGluGlyMetAsnLeuThrTrpTyrArgGluS

          1150                1170                1190
1141 GCAAAGAACCCGTGAACCCGGGCCCTTTGAACAAGAAGGATCACTTCAATGGGACGATCA 1200
     erLysGluProValAsnProGlyProLeuAsnLysLysAspHisPheAsnGlyThrIleT

          1210                1230                1250
1201 CAGTCACGTCTACCCTGCCAGTGAACACCAATGACTGGATCGAGGGCGAGACCTACTATT 1260
     hrValThrSerThrLeuProValAsnThrAsnAspTrpIleGluGlyGluThrTyrTyrC

          1270                1290                1310
1261 GCAGGGTGACCCACCCGCACCTGCCCAAGGACATCGTGCGCTCCATTGCCAAGGCCCCTG 1320
     ysArgValThrHisProHisLeuProLysAspIleValArgSerIleAlaLysAlaProG

          1330                1350                1370
1321 GTGAGCCACGGGCCCAGGGGAGGTGGGCGGGCCTCCTGAGCCGGAGCCTGGGCTGACCCC 1380

          1390                1410                1430
1381 ACACCTATCCACAGGCAAGCGTGCCCCCCCGGATGTGTACTTGTTCCTGCCACCGGAGGA 1440
                    lyLysArgAlaProProAspValTyrLeuPheLeuProProGluGl

          1450                1470                1490
1441 GGAGCAGGGGACCAAGGACAGAGTCACCCTCACGTGCCTGATCCAGAACTTCTTCCCCGC 1500
     uGluGlnGlyThrLysAspArgValThrLeuThrCysLeuIleGlnAsnPhePheProAl
```

FIG.1C

```
          1510                1530                1550
1501 GGACATTTCAGTGCAATGGCTGCGAAACGACAGCCCCATCCAGACAGACCAGTACACCAC 1560
     aAspIleSerValGlnTrpLeuArgAsnAspSerProIleGlnThrAspGlnTyrThrTh

          1570                1590                1610
1561 CACGGGGCCCCACAAGGTCTCGGGCTCCAGGCCTGCCTTCTTCATCTTCAGCCGCCTGGA 1620
     rThrGlyProHisLysValSerGlySerArgProAlaPhePheIlePheSerArgLeuGl

          1630                1650                1670
1621 GGTTAGCCGGGTGGACTGGGAGCAGAAAAACAAATTCACCTGCCAAGTGGTGCATGAGGC 1680
     uValSerArgValAspTrpGluGlnLysAsnLysPheThrCysGlnValValHisGluAl

          1690                1710                1730
1681 GCTGTCCGGCTCTAGGATCCTCCAGAAATGGGTGTCCAAAACCCCCGGTAAATGATGCCC 1740
     aLeuSerGlySerArgIleLeuGlnLysTrpValSerLysThrProGlyLys

          1750                1770                1790
1741 ACCCTCCTCCCGCCGCCACCCCCCAGGGCTCCACCTGCTGGGAGGGAGGGGGGCTGGCAA 1800

          1810                1830                1850
1801 GACCCTCCATCTGTCCTTGTCAATAAACACTCCAGTGTCTGCTTGGAGCCCTGGGCACAC 1860

          1870                1890                1910
1861 CCATTTCTTGGGGGTGGGCAGGGTTGCAGAGCAGGGATGTCTTGGCACAGAAGGGTCCCC 1920

1921 CAGGGTGT 1928
```

FIG.1D

% IDENTITY OF CANINE IgεTO IgεOF OTHER SPECIES					
	CH1	CH2	CH3	CH4	TOTAL
MOUSE IgεDNA	54	63	64	66	62
MOUSE IgεPROTEIN	42	42	55	56	49
HUMAN Igε1 DNA	69	67	74	71	70
HUMAN Igε1 PROTEIN	59	53	62	55	57

FIG.2

% IDENTITY OF CANINE IgαTO IgαOF OTHER SPECIES				
	CH1	CH2	CH3	TOTAL
MOUSE IgαDNA	59	73	78	71
MOUSE IgαPROTEIN	52	67	73	65
HUMAN Igα1 DNA	72	74	83	76
HUMAN Igα1 PROTEIN	57	70	82	70

FIG.4

```
                10                  30                  50
                .                   .                   .
  1 AGTGACCTAGCGTGTCATTCTGACCCAGGTCTCGGCATATGAACTGCATGACCTTGGGCT  60

                70                  90                  110
                .                   .                   .
 61 GTCACTGACCATCTCTATGCAGTTTCCTCTAGTGCAAAGAAAAAATAGCCCTCACCCTGC 120

                130                 150                 170
                .                   .                   .
121 CTGTGAGGCCATGTAAGGGGTCCAGACAGCACTGGCCCACCAGCTCACAGAGTGTCCTGT 180

                190                 210                 230
                .                   .                   .
181 GTCACAGAGTCCAAAACCAGCCCCAGTGTGTTCCCGCTGAGCCTCTGCCACCAGGAGTCA 240
        XXSerLysThrSerProSerValPheProLeuSerLeuCysHisGlnGluSer
                                    .
                250                 270                 290
                .                   .                   .
241 GAAGGGTACGTGGTCATCGGCTGCCTGGTGCAGGGATTCTTCCCACCGGAGCCTGTGAAC 300
    GluGlyTyrValValIleGlyCysLeuValGlnGlyPhePheProProGluProValAsn

                310                 330                 350
                .                   .                   .
301 GTGACCTGGAATGCCGGCAAGGACAGCACATCTGTCAAGAACTTCCCCCCCATGAAGGCT 360
    ValThrTrpAsnAlaGlyLysAspSerThrSerValLysAsnPheProProMetLysAla

                370                 390                 410
                .                   .                   .
361 GCTACCGGAAGCCTATACACCATGAGCAGCCAGTTGACCCTGCCAGCCGCCCAGTGCCCT 420
    AlaThrGlySerLeuTyrThrMetSerSerGlnLeuThrLeuProAlaAlaGlnCysPro

                430                 450                 470
                .                   .                   .
421 GATGACTCGTCTGTGAAATGCCAAGTGCAGCATGCTTCCAGCCCCAGCAAGGCAGTGTCT 480
    AspAspSerSerValLysCysGlnValGlnHisAlaSerSerProSerLysAlaValSer

                490                 510                 530
                .                   .                   .
481 GTGCCCTGCAAAGGTCAGAGGGCAGGCTGGGGAGGGGCAGGGGCCCCACATCCTCACTCT 540
```

```
          550                 570                 590

541 GACCCTCCACTTGGAGTTCTGGCCCCAAGGACACTCCACGGGGAGGACAGTGGGCTGCTG 600

          610                 630                 650

601 GGCTGAGCTCCCAGCAAGTGGCCAAGGTGGGGCCTCCATGAAGGACCTGGAGGGTGGCAG 660

          670                 690                 710

661 GGGGCAGGCAGGCAGAGGGTGCACACTGACCTGTTCCAATCTCTCTCTCTCTCTCTCTCT 720

          730                 750                 770

721 CTCTCTCTGCTCCTGAAGATAACAGTCATCCGTGTCATCCATGTCCCTCGTGCAATGAGC 780
                    spAsnSerHisProCysHisProCysProSerCysAsnGluP
                    |                             |
          790                 810                 830

781 CCCGCCTGTCACTACAGAAGCCAGCCCTCGAGGATCTGCTTTTAGGCTCCAATGCCAGCC 840
    roArgLeuSerLeuGlnLysProAlaLeuGluAspLeuLeuLeuGlySerAsnAlaSerL

          850                 870                 890

841 TCACATGCACACTGAGTGGCCTGAAAGACCCCAAGGGTGCCACCTTCACCTGGAACCCCT 900
    euThrCysThrLeuSerGlyLeuLysAspProLysGlyAlaThrPheThrTrpAsnProS

          910                 930                 950

901 CCAAAGGGAAGGAACCCATCCAGAAGAATCCTGAGCGTGACTCCTGTGGCTGCTACAGTG 960
    erLysGlyLysGluProIleGlnLysAsnProGluArgAspSerCysGlyCysTyrSerV

          970                 990                 1010

961 TGTCCAGTGTCCTACCAGGCTGTGCTGATCCATGGAACCATGGGGACACCTTCTCCTGCA 1020
    alSerSerValLeuProGlyCysAlaAspProTrpAsnHisGlyAspThrPheSerCysT

          1030                1050                1070

1021 CAGCCACCCACCCTGAATCCAAGAGCCCGATCACTGTCAGCATCACCAAAACCACAGGTG 1080
     hrAlaThrHisProGluSerLysSerProIleThrValSerIleThrLysThrThrG
```

FIG.3B

1090 1110 1130

1081 GGCCCAGACCCTGCCCGTGAGGCACTGCTTGGCACACAAAAGTTTGTGAGGCAACTCCTA 1140

1150 1170 1190

1141 AGCCTGCTTCCTTCCTCTAGCCCCTGGGCTTGGGTGCTCCCACCCACATTTTACAAAGGG 1200

1210 1230 1250

1201 AAACTGTGGCATGGGGTGCTATGGGGAAGAAGGCTCTTCCCCCACCCCAGATCCCTGACC 1260

1270 1290 1310

1261 TGGCTCTCTGTCCTGCAGAGCACATCCCGCCCCAGGTCCACCTGCTGCCGCCGCCGTCGG 1320
luHisIleProProGlnValHisLeuLeuProProProSerG

1330 1350 1370

1321 AAGAGCTGGCCCTCAATGAGCTGGTGACACTGACGTGCTTGGTGAGGGGCTTCAAACCAA 1380
luGluLeuAlaLeuAsnGluLeuValThrLeuThrCysLeuValArgGlyPheLysProL

1390 1410 1430

1381 AAGATGTGCTCGTACGATGGCTGCAAGGGACCCAGGAGCTACCCCAAGAGAAGTACTTGA 1440
ysAspValLeuValArgTrpLeuGlnGlyThrGlnGluLeuProGlnGluLysTyrLeuT

1450 1470 1490

1441 CCTGGGAGCCCCTGAAGGAGCCTGACCAGACCAACATGTTTGCCGTGACCAGCATGCTGA 1500
hrTrpGluProLeuLysGluProAspGlnThrAsnMetPheAlaValThrSerMetLeuA

1510 1530 1550

1501 GGGTGACAGCCGAAGACTGGAAGCAGGGGGAGAAGTTCTCCTGCATGGTGGGCCACGAGG 1560
rgValThrAlaGluAspTrpLysGlnGlyGluLysPheSerCysMetValGlyHisGluA

1570 1590 1610

1561 CTCTGCCCATGTCCTTCACCCAGAAGACCATCGACCGCCTGGCGGGTAAACCCACCCACG 1620
laLeuProMetSerPheThrGlnLysThrIleAspArgLeuAlaGlyLysProThrHisV

```
          1630                1650                1670
           .   .   .   .   .   .   .   .   .   .   .   .
1621  TCAACGTGTCTGTGGTCATGGCAGAGGTGGACGGCATCTGCTACTAAACCGCCCAATCTT  1680
      alAsnValSerValValMetAlaGluValAspGlyIleCysTyr

          1690                1710                1730
           .   .   .   .   .   .   .   .   .   .   .   .
1681  CCCTCCCTAAATAAACTCCATGCTTGCCCAAAGCAGCCCCGTGCTTCCATCAGGCCGCCT  1740

          1750                1770
           .   .   .   .   .   .   .   .
1741  GTCTGTCCATATTCGGGGTCTGTGGCATACTGAGGCAGGGGTAGAGCTC  1789
```

FIG.3D

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/13795

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 39/395; C12P 21/04; C07H 21/02
US CL :424/133.1; 435/69.6; 536/23.53
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/133.1; 435/69.6; 536/23.53

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

BIOSIS, MEDLINE, and HCA (chem abs)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO, A, 94/21676 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 29 September 1994, see entire document.	1-9, 14, 20

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be part of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 MARCH 1996	25 MAR 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer T. NISBET

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/13795

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-9, 14, 20

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/13795

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-9, 14 and 20, drawn to a first appearing product comprising a DNA sequence encoding canine immunoglobulin E and a recombinant method of using said DNA to make the corresponding protein.

Group II, claim(s) 10-13, drawn to the corresponding IgE antibody protein which is a separate, second product from that set forth in Group I.

Group III, claim(s) 15 and 18, drawn to a method of using the anti-IgE antibodie modulating compounds in treating patients, and a method of making said anti-IgE.

Group IV, claims 16 and 17, drawn to a third appearing product which is an anti-idiotypic antibody against canine IgE protein.

Group V, claim 19, drawn to a fourth appearing product which is DNA encoding a canine IgA antibody protein.

The inventions listed as Groups I & V and II & III & IV do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the two products are separate molecules which have separate structures. Specifically, DNA of Groups I and V, is a nucleic acid which is a polymer of 4, distinct, basic nucleotide subunits. In contrast, the proteins of Group II are distinct as a separate class of molecules comprising amino acid subunits. These two classes of molecules have separate properties, are capable of separate manufacture, and have separate uses. Therefore, the products of Groups I & V and II & III & IV are not so linked as to form a single general inventive concept.

The inventions of Group II and Group III are not so linked as to relate to a single inventive concept because Group III is a method of using the product of Group IV (third product).

The inventions of Group II and Group IV are not so linked as to relate to a single inventive concept because the two groups relate to two different kinds of antibodies. The invention of Group IV has a binding specificity to a different antibody. Therefore, the invention of Group IV is an anti-idiotypic antibody. In contrast, the invention of Group II is different because it has the effect of mediating allergic reactions in dogs (canines). Since the specificity of the antibodies is different,the function of these antibodies isdifferent.Consequently, the inventions are fundamentally different resulting in lack of unity.

The inventions of Group III and Group IV are not so linked as to relate to a single inventive concept because the invention of Group III is a separate method of using the products of Group IV, and the product of Group IV is not the first appearing product or main invention. See PCT Rule 13 and the Administrative Instructions, Annex B.

The inventions of Groups I and V are not so linked as to form a single inventive concept. The two different antibodies are of a different subclass. The primary focus of applicant's invention as far as pages 1-3 of applicant's disclosure is concerned revolves around the particular effects of the constant region of the antibody. The invention set forth in Group V deals with an antibody of the a different constant region, namely IgA. Consequently, as far as the fundamental aspect of the invention is concerned, i.e. the properties of the constant region, the two Groups deal with fundamentally different concepts. Namely, Group I deals with the effects and sequence of IgE while Group V deals with the effects of IgA. Since the effects of IgA and IgE are distinct and the sequences are different, both the structure and function of Groups I and V are separate and thus lack unity.